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Formulation of a Nucleopolyhedrovirus with Boric Acid for Control of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Maize

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The degree of control of the fall armyworm, *Spodoptera frugiperda*, by a multiple nucleopolyhedrovirus (SfMNPV) appears to be limited by the quantity of inoculum consumed by the insect and the delivery of the virus to the insect feeding site. The formulation of the virus with phagostimulants and/or viral synergists, such as boric acid, may help overcome this problem. The present study aimed to determine the degree of potentiation of boric acid toward SfMNPV in a granular phagostimulant formulation. In a laboratory bioassay the LC_{50} value for second-instar larvae was reduced from 114 virus occlusion bodies (OBs)/mm^2 of diet surface for virus alone to 51 OBs/mm^2 of diet in the presence of 1% boric acid. The mean time to death of larvae exposed to virus mixed with 0.5 or 1% boric acid was not significantly different from that of larvae inoculated with virus alone. Increasing the concentration of boric acid at a single determined concentration of virus (80 OBs/mm^2) resulted in a significant increase in the prevalence of virus-induced mortality. The boric acid alone did not cause *S. frugiperda* mortality at the concentrations tested. A field trial performed with *S. frugiperda* larvae held on plants within fine gauze bags indicated that application of maize flour granules containing virus + 1% boric acid caused a significant increase in virus-induced mortality compared to application of granules containing virus alone. A randomized block experiment performed later also resulted in a higher prevalence of virus-induced mortality in *S. frugiperda* larvae exposed to virus mixed with 1% boric acid in samples collected at 5 days postapplication and reared in the laboratory until death or pupation, but not in samples made at 1 day and 3 days postapplication. Differences in the prevalence of virus infection in insects collected at each time point may have been related to the consistency of the granular formulation, which turned into a paste and adhered to the surface of maize plants under conditions of heavy rainfall. Granules containing 1 and 4% boric acid were not toxic to the earwig, *Doru taeniatum*, in the laboratory. The same concentrations of boric acid sprayed onto maize plants did not significantly reduce the abundance of natural enemies or other nontarget insects at any sample time point. Boric acid offers an economical means of enhancing baculovirus activity with little apparent risk to nontarget arthropods. © 2002 Elsevier Science

Key Words: **baculovirus; fall armyworm; potentiation; phagostimulant; nontarget effects.**

INTRODUCTION

In many cases, control of insect pests by baculoviruses is possible by the application of occluded virus suspended in water. However, further formulation may substantially improve the efficiency of the application, the persistence of activity of the virus in the field, and the handling properties and shelf life of the product (Jones *et al.*, 1997). Moreover, adjuvants may be included in the formulation to stimulate consumption of virus inoculum by the pest or enhance the lethal effect of the virus toward the host (Bell and Kanavel, 1975; Burges and Jones, 1998).

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is a major pest of maize and sorghum from the United States south to Argentina (Hruska and Gould, 1997). Conventional control measures involve the application of synthetic insecticides in sprays or granules directly applied to the whorl. However, due to incorrect handling, chronic pesticide intoxication of farmers is reported to be prevalent in Mesoamerica (McConnell and Hruska, 1993; Tinoco and Halperin, 1998). This has motivated a research project aimed at developing a multiple nucleopolyhedrovirus (NPV) (Baculoviridae) of *S. frugiperda*

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(SfMNPV) as a safe biological insecticide for Mesoamerican maize growers (Williams *et al.*, 1999).

The degree of control of *S. frugiperda* appears to be limited by the ability to deliver the inoculum to the feeding site of the insect, i.e., the developing leaf whorl. Spray application of 1.5×10^{12} viral occlusion bodies (OBs)/ha results in approximately 40% pest mortality in larvae sampled at 2 days postapplication and reared in the laboratory until death (Williams *et al.*, 1999). Insect parasitoids active in virus-sprayed plots contribute an additional 15–20% mortality but application of higher concentrations of virus does not substantially improve the degree of pest control (Martínez *et al.*, 2000).

There are two ways in which formulation can overcome this problem: first, the use of phagostimulants to increase the quantity of inoculum consumed by the insect and, second, the use of virus synergists to improve the specific activity of the virus. Recently, granular formulation based on nixtamalized maize flour has been shown to significantly increase the degree of control of lepidopteran pests using *Bacillus thuringiensis* (Bt) Berliner and a nucleopolyhedrovirus (Tamez-Guerra *et al.*, 1998, 2000).

Boric acid (H_3BO_3) has an established history of use for the control of ants and cockroaches (Hayes and Laws, 1991). This compound has been demonstrated to potentiate the activity of *B. thuringiensis* and several baculoviruses (Shapiro and Bell, 1982; Morris *et al.*, 1995). The degree of potentiation increases with the concentration of the acid. For example, the LC_{50} of an NPV of *Anticarsia gemmatalis* (Hübner) was reduced by a factor of approximately fivefold in the presence of 0.045% boric acid. The lethal time (LT_{50}) was also reduced (Morales *et al.*, 1997). Similar results have been reported for the NPVs of *Lymantria dispar* (L.) and *Spodoptera litura* (F.) when mixed with 0.5–1% boric acid (Shapiro and Bell, 1982; Chaudhari, 1992).

Incorporation of boric acid into baculovirus formulations is an attractive proposition as it is inexpensive and has a low mammalian toxicity. The aim of the present study was to determine the degree of potentiation of boric acid toward SfMNPV in a granular phagostimulant formulation. The possible impact of boric acid applications on insect natural enemies was also tested.

METHODS

Bioassay

All laboratory procedures were performed at 25 ± 1°C, 75–85% RH, and 12 h:12 h L:D photoperiod. To determine the degree of synergism provided by different concentrations of boric acid and SfMNPV, a bioassay based on the technique described by Del Rincón-Castro and Ibarra (1997) was performed. The SfMNPV isolate had previously been characterized by Escribano *et al.* (1999). Virus was produced in fourth-instar *S. frugiperda* larvae individually maintained in 25-ml plastic cups containing a semisynthetic diet based on soya and maize without formaldehyde (modified from Mihm, 1984). Virus-killed larvae were triturated in 0.1% (w/v) sodium dodecyl sulfate (SDS) and centrifuged at 90*g* for 5 min, and the supernatant was centrifuged at 3000*g* for 10 min. Pelleted OBs were resuspended in sterile distilled water, counted using a bacterial counting chamber, and stored at 4°C for 24 h prior to use. Sterile plastic petri dishes (9 cm diameter) were half filled with semisynthetic diet. The diet was allowed to solidify and then inoculated with 80 OBs/mm^2 diet surface. Viral OBs had been suspended in 0.5, 1, 2, 3, 4, or 5% (wt/vol) laboratory-grade boric acid solution.

A rectangular plastic grid 70 × 54 mm divided into 12 squares with an internal area of 15 × 15 mm was pressed into the diet to form 12 identical compartments into each of which was placed a second-instar *S. frugiperda* larva taken from the laboratory culture maintained at El Colegio de la Frontera Sur (ECOSUR), Tapachula, Mexico. The grid was then covered with a thin glass slide and the lid of the petri dish. Either 24 or 36 larvae (two to three petri dishes) were exposed to each concentration. A similar number of control larvae were placed in petri dishes containing virus alone or dishes containing neither virus nor boric acid. Larvae were checked twice daily for mortality until 14 days postinoculation by which time survivors had pupated. Viral deaths were confirmed by examination of Giemsa-stained smears of insect cadavers. The bioassay was performed five times.

To determine the effect of boric acid on the viral LC_{50}, an identical experiment was performed using second-instar larvae (36 larvae/treatment) exposed to five concentrations of virus between 20 and 320 OBs/mm^2 mixed with 0, 0.5, or 1% boric acid. An identical number of control larvae were treated similarly, but were not exposed to virus. The experiment was performed three times.

Preparation of Granular Formulation

Phagostimulant granules were prepared by mixing 160 g nixtamalized maize flour, 34 g pregelatinized cornstarch, 16 ml of corn oil, and 200 ml of distilled water to form a paste which was left to stand for 20 min before being passed through a wire gauze with a mesh aperture of 1.2 mm. The resulting granules were placed next to a fan ventilator and allowed to air dry for 16 h at 25 ± 1°C prior to use. For granules containing boric acid and/or virus, the appropriate quantity of boric acid or virus was added to the water component

and mixed thoroughly to ensure homogeneous incorporation, prior to being passed through the wire gauze.

Field trials with these granules have indicated that a suitable application rate is 24 kg/ha (J. Cisneros and T. Williams, unpublished data). Granules that contained 6.25×10^{10} OBs/kg of granules, equivalent to 1.5×10^{12} OBs/ha, which represented a standard application rate determined in previous studies (Martínez *et al.,* 2000) were therefore prepared.

Field Trial: Individual Plants

To test the degree of potentiation of virus activity by boric acid in the field, an experiment was performed during the month of August 1999 in a maize field close to the village of Morelos (14°52′ N: 92°22′ W), approximately 15 km southwest of the town of Tapachula, Chiapas, Mexico. The climatic conditions prevailing in this humid tropical coastal region have been described previously (Williams *et al.,* 1999). Rainfall occurred in the afternoon every day during the experimental period. Maize plants were approximately 45 cm tall, planted at a density of approximately 30,000 plants/ha, and had not been previously treated with insecticides.

Individual plants were randomly selected at intervals of 1–2 m and were manually infested with five second-instar larvae of *S. frugiperda* obtained from the laboratory culture held in ECOSUR. Plants showing evidence of *S. frugiperda* infestation were excluded from the study. Since late-instar larvae are highly cannibalistic, this behavior can affect insect survival and the probability of virus transmission (Chapman *et al.,* 1999). Infested plants were treated with 1.7 ± 0.08 g of granules/plant (mean $\pm$ SE) using a plastic teaspoon dosing technique (Williams *et al.,* 1999). A fine gauze bag was placed over each plant and tied firmly at the base to contain *S. frugiperda* larvae and exclude insect predators and parasitoids. Such bags had been shown to function effectively in a previous study (Castillejos *et al.,* 2001). Granules applied to plants contained 6.25×10^{10} OBs/kg with or without 1% (wt/wt) boric acid, whereas control granules did not contain virus or boric acid. Because bags were sealed isolated experimental units, they represent independent replicates. A total of 90 plants were selected for each treatment.

After 2 days, half of the experimental plants were cut, placed in plastic bags, and transported back to the laboratory where the gauze bags were removed. Living *S. frugiperda* larvae were transferred to individual plastic pots containing semisynthetic diet and reared in the laboratory until pupation or death. Virus deaths were confirmed by microscopic examination of Giemsa-stained smears (Hunter-Fujita *et al.,* 1998). The process was repeated for the remaining half of the experimental plants at 5 days postapplication.

Field Trial: Randomized Blocks

A further field trial was performed in a maize field close to the village of Culiapam de Guerrero, approximately 20 km south of the city of Oaxaca, in Oaxaca State, Mexico, during the month of August 2000. During the study period, the weather in this region was warm (daily range 15–25°C) and semiarid. Maize plants were planted at a density of approximately 25,000 plants/ha and were 50–60 cm tall at the start of the trial. Plants were divided into experimental blocks of 6×5 m with a barrier of 5 m of maize plants between blocks. As in the previous experiment, plants were manually infested with five second-instar *S. frugiperda* larvae from the laboratory culture. Twenty-four hours later, each of the blocks was randomly assigned to one of the following treatments: (i) control granules devoid of virus or boric acid, (ii) a commercial granular insecticide based on chlorpyrifos applied at a rate of 5 kg/ha (Knocker 3G; Bravo S.A. de C.V., Mexico; containing 3% active ingredient), (iii) granules containing 1% boric acid, (iv) granules containing 6.25×10^{10} OBs/kg, or (v) granules containing 6.25×10^{10} OBs/kg + 1% boric acid. The quantities and methods used to apply maize flour granules were as described in the previous experiment. There were six replicate blocks assigned to each treatment.

At 1, 3, and 5 days postapplication, 10 randomly selected plants from each block were cut, placed into plastic bags, and transported to the laboratory, where living *S. frugiperda* larvae were transferred to individual plastic pots containing semisynthetic diet and reared to death or pupation as described previously.

Effect of Boric Acid on Earwigs

Adult earwigs, *Doru taeniatum* Dorhn (Dermaptera: Forficulidae), were collected in maize and sorghum fields around Tapachula, Chiapas, Mexico and were maintained in the laboratory at 26 ± 1°C in ventilated plastic containers containing dampened cotton wool and maize pollen. Earwigs were randomly selected and individually confined in 12-ml ventilated plastic pots with 0.5 g of granules containing 0, 1, or 4% (wt/wt) boric acid. Pots were covered with dampened paper towels and held at 25 ± 1°C, 12 h:12 h photoperiod. Earwigs were checked for mortality after 2 and 7 days. Additional food items were not provided during the experimental period. The experiment was performed four times with 25 earwigs in each treatment ($N = 300$ total).

Field Trial: Effect of Boric Acid on Nontarget Arthropods

A field trial was performed to determine the possible impact of boric acid applications on nontarget insects and spiders. In this case, plants were subjected to

spray applications as sprays tend to have a greater impact on nontarget invertebrates than targeted granular applications (Croft, 1990). The experimental site was a maize field close to the village of Alvaro Obregón, approximately 22 km west of the town of Tapachula, Chiapas, Mexico. Maize plants were planted at a density of approximately 30,000 plants/ha and were 80–100 cm tall at the start of the trial. Part of the field (0.36 ha) was divided into blocks 5 × 6 m with 5 m of maize planted between blocks. Ten randomly selected blocks were assigned to each of the following treatments: (i) water control, (ii) chlorpyrifos (Lorsban 480 EM; Dow AgroScience) at the recommended rate of 0.75 liter/ha, (iii) 1% boric acid, and (iv) 4% boric acid. In all cases the volume of the application was 600 liters/ha and 0.02% Agral Plus (Zeneca) was included as a wetter-sticker.

At 1, 3, and 6 days postapplication, the number of insects present on 10 randomly selected plants in each block was checked and recorded. Sampled plants were never resampled. Insects were classified as natural enemies (earwigs, predatory bugs, predatory Neuroptera, predatory beetles, spiders, syrphid larvae, etc.) or other insects (lepidopteran larvae, phytophagous beetles, thrips, aphid colonies, etc.) as described previously (Williams *et al.*, 1999).

Statistical Analysis

Virus-induced mortality data from bioassays and field experiments were subjected to regression analysis or ANOVA for each time point using GLIM (generalized linear interactive modeling; Numerical Algorithms Group, Oxford, UK) with binomial error structure specified. GLIM presents the results of such analyses in terms of χ^2 statistics. Where necessary, scaling was performed to accommodate minor overdispersion. The results of scaled analyses are presented as F statistics with the scale parameter indicated. Differences between treatment means are presented as t statistics (Crawley, 1993). In all cases, the behavior of models was checked by examination of the distribution of residuals and fitted values. The mean time to death of bioassay insects was calculated using GLIM with a normal error distribution; individuals that did not succumb to virus infection were excluded from the analysis (Farrar and Ridgway, 1998). As the abundance of insect natural enemies and other insects on experimental plants are unlikely to be independent variables, field trail data on the effect of boric acid on nontarget arthropods were subjected to multiple analysis of variance (MANOVA) using SAS (SAS Institute, 1992) with natural enemies and other insects as dependent variables. The significance of treatment effects at each time point and multiple comparisons between treatments were interpreted in terms of F statistics generated from Pillai's trace (Winer, 1971).

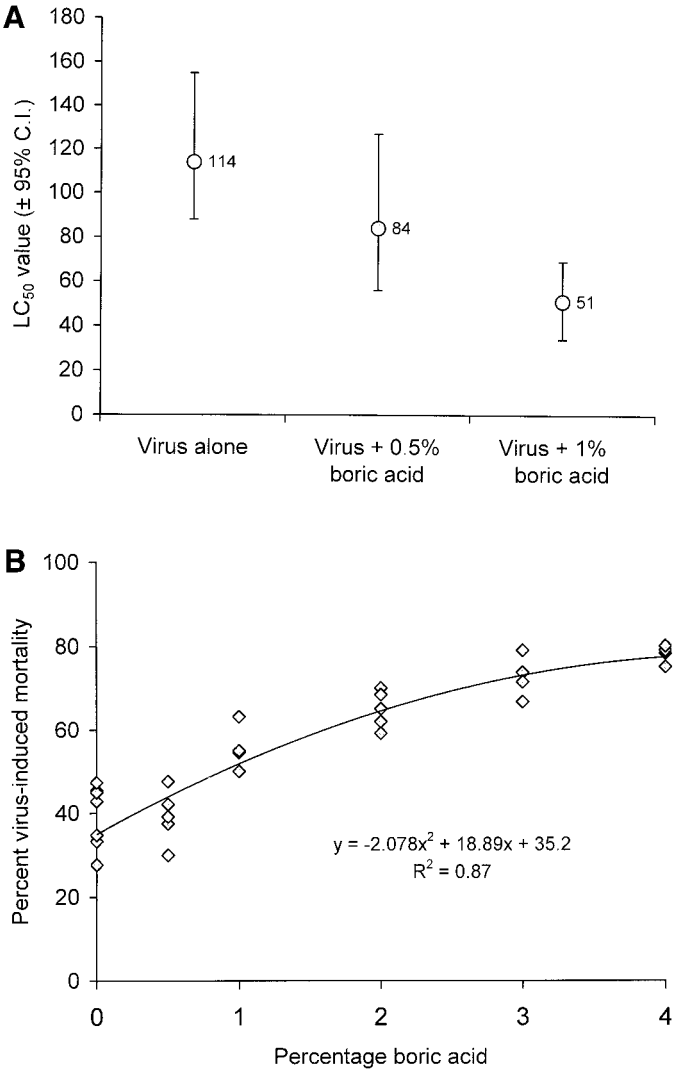

FIG. 1. (A) LC_{50} values for second-instar *Spodoptera frugiperda* larvae exposed to a surface contamination bioassay of nucleopolyhedrovirus alone or mixed with 0.5% or 1% boric acid. Bars indicate 95% confidence limits. (B) Effect of boric acid concentration on virus-induced mortality of second-instar *S. frugiperda* larvae exposed to a single concentration of 80 viral occlusion bodies (OBs)/mm^2 of diet surface.

RESULTS

Bioassay

The LC_{50} value calculated for SfMNPV alone was 114 OBs/mm^2 of diet surface (Fig. 1A). The LC_{50} value was reduced in the presence of 0.5% boric acid, but the effect was not significant. However, the LC_{50} value was reduced to 51 OBs/mm^2 of diet in the presence of 1% boric acid (Fig. 1A), significantly lower than the value for virus alone ($\chi^2 = 19.4$, $df = 2$, $P < 0.001$). Control larvae suffered no virus mortality. The mean time to death of larvae exposed to virus + 0.5 or 1% boric acid was not significantly different from that of larvae in-

oculated with virus alone ($t = 0.08$, $df = 206$, $P = 0.93$).

Increasing the concentration of boric acid at a single determined concentration of virus resulted in an increasing prevalence of *S. frugiperda* mortality. The prevalence of virus-induced mortality at 80 OBs/mm^2 of diet rose from 39.4% in the absence of boric acid to 77.5% in the presence of 4% boric acid ($\chi^2 = 60.6$, $df = 1$, $P < 0.001$) (Fig. 1B). The presence of 0.5–4% boric acid did not result in a significant increase in nonvirus mortality compared to control larvae ($F_{1,28} = 1.56$, $P = 0.22$, scale parameter = 1.26).

Field Trial: Individual Plants

Samples were collected from 39–45 plants/treatment at each time point. At 2 days postapplication, recovery of larvae from bagged plants was similar between all treatments, ranging from 3.2 ± 0.4 larvae/plant (mean ± SE) on plants treated with virus + boric acid to 3.4 ± 0.4 larvae/plant on plants treated with virus alone ($F_{2,133} = 0.13$, $P = 0.87$). Similarly, at 5 days postapplication, there were no significant differences in larval recovery between treatments which ranged from 3.8 ± 0.3 larvae/plant in the controls to 5.4 ± 1.1 larvae/plant in the virus + boric acid treatment ($F_{2,122} = 2.67$, $P = 0.07$), indicating that some of the experimental plants had a natural infestation of *S. frugiperda* eggs or early instar larvae that was not evident at the start of the experiment.

Compared to granules containing virus alone, application of granules containing virus + boric acid caused a significant increase in virus-induced mortality in larvae collected at 2 days postapplication and reared in the laboratory until death or pupation ($F_{1,87} = 8.17$, $P = 0.005$, scale parameter = 1.21) and similarly at 5 days postapplication ($F_{1,82} = 4.84$, $P = 0.03$, scale parameter = 1.34) (Fig. 2A). Only one parasitoid emerged in larvae sampled in this study, probably because of parasitoid exclusion by the fine gauze bags used to contain larvae and exclude natural enemies. No virus mortality was observed in insects recovered from plants treated with control granules.

Field Trial: Randomized Blocks

Total recovery of larvae was significantly reduced in insecticide-treated plots (total 6 larvae) but was statistically similar among other treatments ranging from 26 to 182 larvae per treatment per sample ($F_{4,25} = 14.2$; $P < 0.001$). Importantly, the mean (±SE) recovery of larvae from plants treated with granules containing boric acid alone (9.2 ± 1.5 larvae/sample/block) was not significantly different from the recovery of larvae from plants treated with control granules (11.8 ± 2.4 larvae/sample/block), indicating that boric acid per se did not reduce *S. frugiperda* survival ($t = 0.79$, $df = 9$, $P = 0.45$).

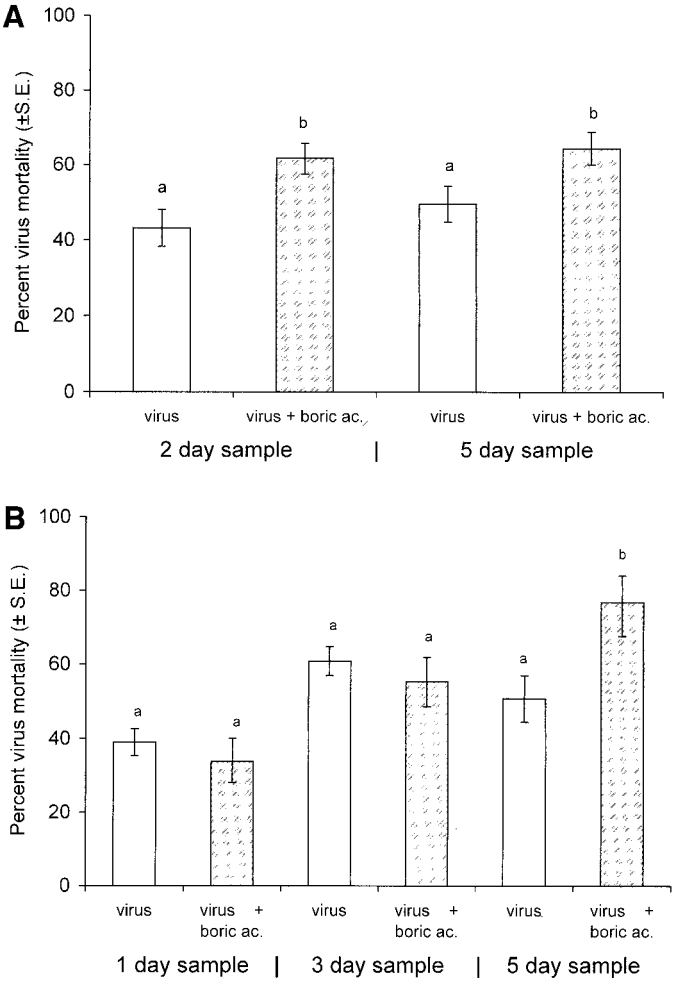

FIG. 2. Percentage virus-induced mortality in *S. frugiperda* larvae exposed to maize flour granules containing 6.25×10^{10} OBs/kg of virus alone or mixed with 1% (wt/wt) boric acid. (A) Field trial in Alvaro Obregón, Chiapas, Mexico in which larvae were recovered from individual bagged plants at 2 and 5 days postapplication and reared on semisynthetic diet in the laboratory until death or pupation. (B) Field trial in Culiapam de Guerrero, Oaxaca, Mexico using a randomized block design. Fall armyworm larvae were recovered from 10 plants/block at 1, 3, and 5 days postapplication and reared on semisynthetic diet in the laboratory until death or pupation. No virus mortality occurred in larvae recovered from plants treated with control granules or granules containing boric acid alone. In all cases, columns bearing the same letter are not significantly different for comparisons within each sample (time point) (GLIM, binomial errors, $P > 0.05$). Virus-induced mortality was not observed in larvae collected from control plants in any sample.

The prevalence of virus-induced mortality was not significantly different between virus and virus + boric acid treatments in larvae taken at 1 day ($\chi^2 = 0.63$; $df = 1$; $P = 0.43$) or 3 days ($\chi^2 = 0.72$; $df = 1$; $P = 0.40$) postapplication and reared in the laboratory until death or pupation (Fig. 2B). However, virus mortality was significantly greater in larvae collected from the virus + boric acid treatment at 5 days postappli-

cation and subsequently reared in the laboratory (χ^2 = 5.15, df = 1, P = 0.02). No virus mortality was observed in larvae sampled from the control plants or those treated with granules containing boric acid alone.

The prevalence of parasitoid emergence ranged from 1.7% in larvae sampled from virus-treated plants to 3.6% in larvae collected from plants treated with granules containing boric acid alone, but numbers were too low to permit reliable analysis. No parasitism was observed in larvae from insecticide-treated plants.

Effect of Boric Acid on Earwigs

Of the *D. taeniatum* adults fed maize flour granules containing boric acid, none (0/100) died in the 1% boric acid treatment, 4/100 died in the 4% boric acid treatment, and 1/100 died in the controls after a period of 7 days exposure. Mortality was too low for statistical analysis. Boric acid, therefore, appears not to be orally toxic to earwigs at the concentrations tested.

Field Trial: Effect of Boric Acid on Nontarget Arthropods

The abundance of natural enemies is not independent of the abundance of other insects, especially potential prey on maize plants (Chapman *et al.,* 2000). Therefore, the effect of spray applications of boric acid was compared to control (water) and conventional synthetic insecticide (chlorpyrifos) applications in terms of the number of natural enemies and other insects as dependent variables within a MANOVA analysis. Application of chlorpyrifos caused a significant decrease in the mean number of arthropods (natural enemies and other insects) at 1 day ($F_{2,35}$ = 10.6, $P < 0.001$), 3 days ($F_{2,35}$ = 5.21, P = 0.01), and 6 days ($F_{2,35}$ = 4.28, P = 0.02) postapplication compared to the abundance of insects on control plants (Figs. 3A and 3B). In contrast, application of 1% boric acid caused an apparent reduction in the abundance of natural enemies at 1 day postapplication, but the effect was not significant ($F_{2,35}$ = 0.61, P = 0.55). By 3 days postapplication, the abundance of arthropods was significantly higher than that on control plants at 3 days postapplication ($F_{2,35}$ = 4.85, P = 0.01), mainly due to an abundance of earwigs on plants treated with 1% boric acid. There were no significant differences in the abundance of arthropods on controls and 1% boric acid-treated plants at 6 days postapplication ($F_{2,35}$ = 0.89, P = 0.41). Application of 4% boric acid did not significantly affect arthropod numbers at 1 day ($F_{2,35}$ = 0.51, P = 0.60), 3 days ($F_{2,35}$ = 0.83, P = 0.44), or 6 days ($F_{2,35}$ = 0.21, P = 0.81) postapplication compared to control plants (Figs. 3A and 3B).

DISCUSSION

A 1% solution of boric acid reduced the lethal concentration of SfMNPV in second-instar *S. frugiperda* larvae by a factor of 2.2. This is somewhat less than that reported in some other studies. Shapiro and Bell (1982) reported a 2-fold reduction in LC_{50} of a NPV of *L. dispar* in the presence of 0.5% boric acid and a 7- to 11-fold decrease in the presence of 1% boric acid. Similarly, Morales *et al.* (1997) detected a 5-fold reduction in the LC_{50} of the NPV of *A. gemmatalis* in the presence of 0.045% boric acid incorporated into the diet. The lesser degree of potentiation observed in our study may be related to the duration of exposure and the dose of boric acid consumed by *S. frugiperda* larvae. Fall armyworm larvae have the tendency to eat into the semisynthetic diet and feed beneath the surface. Once the insect has eaten through the contaminated surface layer, the rate and the amount of boric acid consumed during the first few days of the bioassay may have been reduced. Presumably early stage larvae are more susceptible to the potentiating effect of this inorganic acid, but as insects in all treatments were treated identically, the technique of surface contamination bioassay remains useful for comparative studies of this type.

No evidence of direct boric acid toxicity toward *S. frugiperda* larvae was detected at the highest concentration (4%) employed in this study, although preliminary tests with 5 and 6% boric acid solutions did cause mortality in second-instar larvae. In contrast, Shapiro and Bell (1982) reported 25–100% mortality of *L. dispar* larvae exposed to 2.5–10% boric acid. This again may be related to differences in species susceptibility to boric acid or to differences in the feeding habits of *S. frugiperda* larvae compared to *L. dispar.*

The ability of boric acid to enhance virus infectivity was also demonstrated in field studies using a phagostimulant granular formulation based on maize flour. The mixture of virus + 1% boric acid in the field resulted in 15–25% higher prevalence of viral infection than that observed in insects exposed to virus alone. Interestingly, the pattern of infection over time was different from that observed in previous field studies wherein the inocula were applied in an aqueous spray formulation. The prevalence of infection peaks in insects recovered after 1 or 2 days postapplication and reared in the laboratory until death or pupation, but declines rapidly thereafter. This is assumed to be due to loss of viral inocula by UV-inactivation and plant growth dilution. In contrast, the granular formulation persists in the whorl of the plant for periods of several days and fall armyworm larvae were often observed feeding directly on the remains of the granules.

The difference between the two field experiments in the prevalence of virus infections over time may also be related to the persistence of the granular formulation. Rainfall occurred every afternoon during the experi-

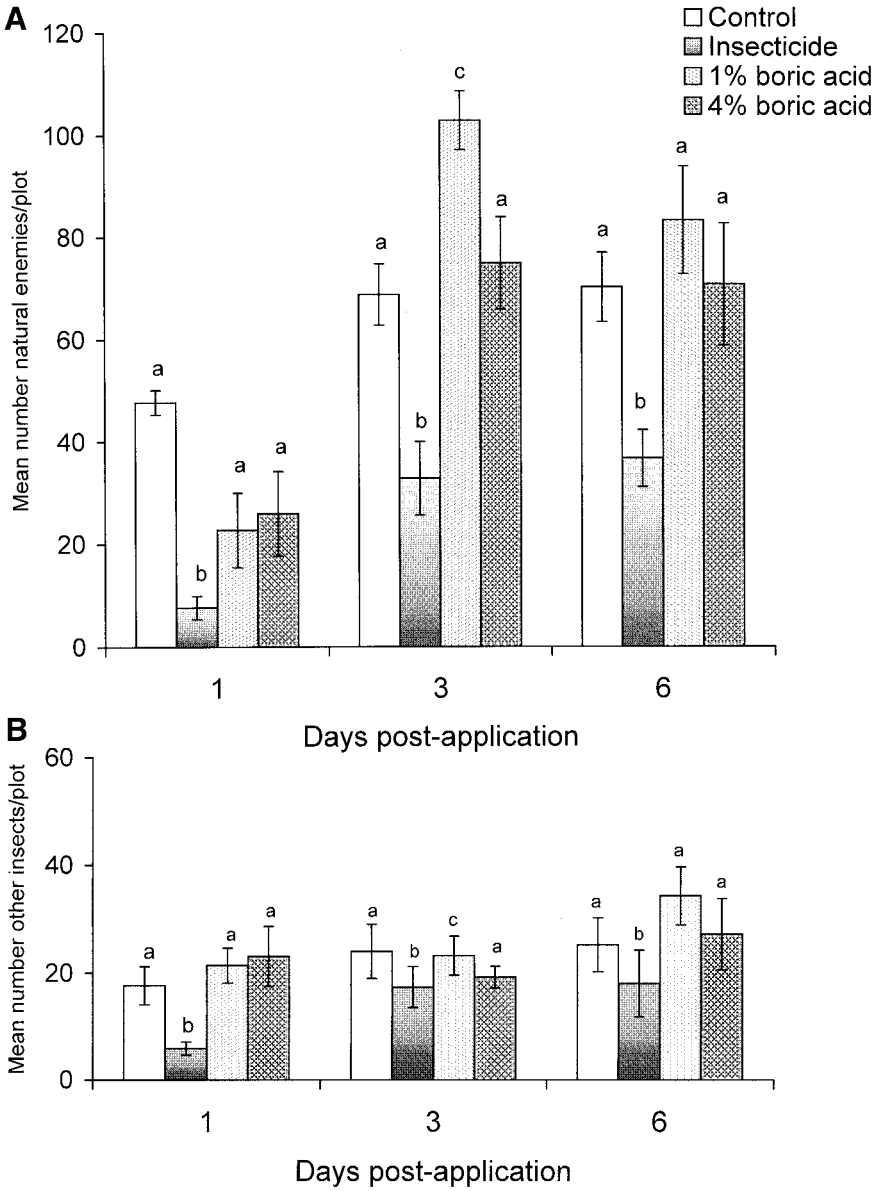

FIG. 3. Effect of boric acid (1 and 4% wt/vol), chlorpyrifos, or water (control) sprayed onto maize plants on the abundance of insect natural enemies and other nontarget insects. Evaluations were conducted at 1, 3, and 6 days postapplication. (A) Natural enemies and (B) other insects were treated as dependent variables in separate MANOVA analyses for each evaluation. Columns labeled with the same letter are not significantly different for comparisons within each evaluation time point. The multiple comparison procedure applies as much to natural enemies as to other insects and because of this the labels on each graph are identical (MANOVA, F generated by Pillai's trace $P > 0.05$).

ment with bagged plants and the granules quickly turned into a paste that adhered to leaf surfaces but remained very attractive to *S. frugiperda* larvae. In contrast, no rain fell during the randomized block experiment in Oaxaca and the formulation only began to lose its granular consistency toward the end of the sampling period. The consumption of dry granules may therefore have been lower during the early stages of the second experiment, resulting in the apparent absence of boric acid potentiation in the larvae recovered at 1 and 3 days and reared in the laboratory until death or pupation. Maize flour and lignin-based formulations have recently been reported to significantly enhance the persistence of an NPV of *Anagrapha falcifara* (Kirby) on cotton leaves following exposure to both simulated rainfall and simulated sunlight (Tamez-Guerra *et al.*, 2000). These aspects of the stability of the phagostimulant formulation merit additional laboratory and field studies.

Despite the fact that the potentiating effect of boric acid on baculoviruses has been recognized for several decades (Yadava, 1970), there have been very few field

studies to evaluate virus + boric acid formulations. In India, Bujjur *et al.* (1991) reported a fourfold improvement in control of *Helicoverpa armigera* (Hübner) on sunflower treated with HaNPV + 0.5% boric acid and Chundurwar *et al.* (1990) reported improved control of *H. armigera* on chickpea treated with HaNPV + 0.5% boric acid.

Boric acid also potentiates the activity of *B. thuringiensis* in Lepidoptera to a degree similar to that observed for baculoviruses (Doane and Wallis, 1964; Govindarajan *et al.*, 1976; Morris *et al.*, 1995). As both Bt and baculoviruses are active by ingestion, we assume that boric acid affects the conditions in the insect gut, possibly by altering the integrity or permeability of the peritrophic membrane or the cells of the gut epidermis. Alternatively, the toxicity of boric acid may cause physiological stress in the insect, increasing its susceptibility to virus infection (Yadava, 1970; Shapiro and Bell, 1982).

Reassuringly, 1 and 4% boric acid caused virtually no mortality in 7-day laboratory feeding tests with earwigs, a species recognized to be an important predator of *S. frugiperda* eggs and larvae in maize (Van Huis, 1981; Jones *et al.*, 1989). Moreover, application of boric acid in aqueous sprays designed to give maximal coverage of maize plants caused no significant reductions in the abundance of insect natural enemies or other nontarget insects at any time point, suggesting that it is compatible for baculovirus formulations at the concentrations tested. This was an issue that was considered important to confirm in field trials, given that boric acid has an established history as a domestic insecticide for control of ants and cockroaches (Walter, 1918; Metcalf and Flint, 1951; Wright and Hillmann, 1973).

The degree of virus potentiation by boric acid is clearly less impressive than that observed with optical brighteners such as Tinopal LPW or Blankophor BBH (Shapiro and Dougherty, 1994; Hamm, 1999) that disrupt the peritrophic membrane of the insect by interfering with chitin-binding proteins essential to membrane integrity (Wang and Granados, 2000). However, at a cost of less than US$2.00/kg in Mexico, the cost of including 1% boric acid in the granular formulation that we used would be around 48 cents/ha. This is far cheaper than using an optical brightener, which is about 20 times more expensive than boric acid. Moreover, one optical brightener has recently been shown to affect the behavior of pollinators that use UV cues to locate and select flowers (Goulson *et al.*, 2000). For the moment, however, the potential indirect effects of boric acid in virus formulations toward pollinators or soil fauna remain unknown.

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